

AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions and listings of claims in the application.

Listing of claims

1-17. (Cancelled)

18. (Currently amended) A method of preparing a proliferation-regulated recombinant adenoviral vector, comprising the steps of: preparing a proliferation-regulated plasmid by[[,]] (a) preparing an expression cassette ~~restriction enzyme-recognizing unit~~ in a plasmid that includes, in order from upstream to downstream, an E1A region, at least one protein-coding region in a E1B region or the entire E1B region, a poly(A) signal sequence, and a recombinase-recognizing sequence, by replacing [[both]] an endogenous promoter in the E1A region and an endogenous promoter regulating expression of [[the]] a protein-coding gene in the at least one protein-coding region in the E1B region with a first and a second multiple cloning site, respectively ~~restriction enzyme-recognizing sequences~~, and (b) introducing a first and a second promoter, each of which expresses[[ing]] specifically in a target organ, into the first and second multiple cloning sites, respectively ~~restriction enzyme-recognizing unit~~; and integrating the proliferation-regulated plasmid into a plasmid containing an E1 region-deleted adenoviral genome.

19. (Previously presented) The method of claim 18, wherein the E1A region lacks a Rb protein-binding sequence.

20. (Currently amended) The method of claim 19, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region ~~and/or a 55KDa protein-coding region~~.

21. (Currently amended) The method of claim 18, wherein the ~~restriction enzyme-recognizing sequences~~ first or the second multiple cloning site includes a blunt-end restriction enzyme site.

22. (Previously presented) The method of claim 18, wherein the recombinase-recognizing sequence is LoxP, LoxH, or a mutant sequence thereof.

23. (Currently amended) A method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene, comprising the steps of: (a) preparing a proliferation-regulated plasmid by (i) preparing an expression cassette ~~restriction enzyme-recognizing unit~~ in a plasmid that includes, in order from upstream to downstream, an E1A region, at least one protein-coding region in a E1B region or the entire E1B region, a poly(A) signal sequence, and a recombinase-recognizing sequence, by replacing ~~[[both]]~~ an endogenous promoter in the E1A region and an endogenous promoter regulating expression of ~~[[the]]~~ a protein-coding gene in the at least one protein-coding region in the E1B region with a first and a second multiple cloning site, respectively ~~restriction enzyme-recognizing sequences~~, and (ii) introducing a first and a second promoter, each of which ~~expresses~~~~[[ing]]~~ specifically in a target organ, into the first and second multiple cloning sites, respectively ~~restriction enzyme-recognizing unit~~; (b) preparing a first therapeutic gene-expressing plasmid by (i) preparing a therapeutic gene ~~-expressing~~ expression cassette ~~[[unit]]~~ by inserting into a plasmid in order from upstream to downstream a recombinase-recognizing sequence and a third multiple cloning site ~~restriction enzyme-recognizing sequence~~, (ii) inserting, in order from upstream to downstream, a constitutive ~~high-expression~~ strong promoter or a therapeutic gene-expressing promoter and a therapeutic gene into the third multiple cloning site ~~restriction enzyme-recognizing sequence of the therapeutic gene-expressing unit~~; (c) preparing a second therapeutic gene-expressing plasmid by allowing a recombinase to react with the proliferation-regulated plasmid and the first therapeutic

gene-expressing plasmid; and (d) integrating the second therapeutic gene-expressing plasmid into a plasmid containing an E1 region-deleted adenoviral genome.

24. (Previously presented) The method of claim 23, wherein the E1A region lacks a Rb protein-binding sequence.

25. (Currently amended) The method of claim 24, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region ~~and/or a 55KDa protein-coding region.~~

26. (Currently amended) A method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene, comprising the steps of: (a) preparing a proliferation-regulated adenoviral vector plasmid by (i) ~~preparing obtaining~~ a proliferation-regulated plasmid by preparing an expression cassette ~~restriction enzyme-recognizing unit~~ in a plasmid that includes, in order from upstream to downstream, an E1A region, at least one protein-coding region in a E1B region or the entire E1B region, a poly(A) signal sequence, and a recombinase-recognizing sequence, by replacing ~~[[both]]~~ an endogenous promoter in the E1A region and an endogenous promoter regulating expression of ~~[[the]]~~ a protein-coding gene in the at least one protein-coding region in the E1B region with a first and a second multiple cloning site, respectively ~~restriction enzyme-recognizing sequences~~, and introducing a first and a second promoter, each of which ~~expresses~~expresses~~[[ing]]~~ specifically in a target organ, into the first and second multiple cloning sites, respectively ~~restriction enzyme-recognizing unit~~, and (ii) integrating the proliferation-regulated plasmid into a plasmid containing an E1 region-deleted adenoviral genome; (b) preparing a therapeutic gene-expressing plasmid by (i) preparing a therapeutic gene ~~-expressing~~ expression cassette ~~[[unit]]~~ by inserting into a plasmid in order from upstream to downstream a recombinase-recognizing sequence and a third multiple cloning site ~~restriction enzyme-recognizing sequence~~, (ii) inserting, in order from upstream to downstream, a constitutive ~~high-expression~~ strong

promoter or a therapeutic gene-expressing promoter and a therapeutic gene into the third multiple cloning site ~~restriction enzyme recognizing sequence of the therapeutic gene-expressing unit~~; and (c) allowing a recombinase to react with the proliferation-regulated adenoviral vector plasmid and the therapeutic gene-expressing plasmid.

27. (Previously presented) The method of claim 26, wherein the E1A region lacks a Rb protein-binding sequence.

28. (Currently amended) The method of claim 27, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region ~~and/or a 55KDa protein-coding region~~.

29. (Currently amended) The method of claim 26, ~~further comprising the steps of:~~ wherein step (c) is accomplished by mixing the proliferation-regulated adenoviral vector of step (a) plasmid and the first proliferation-regulated adenoviral therapeutic gene-expressing plasmid of step (b), allowing a recombinase to react with the mixture, and then, transforming the ~~plasmids~~ mixture into a cell ~~each other~~.

30. (Currently amended) The method of claim 26, ~~further comprising the steps of:~~ wherein step (c) is accomplished by cotransfecting the proliferation-regulated adenoviral vector of step (a) plasmid and the therapeutic gene-expressing plasmid of step (b) into a recombinase-expressing cell.

31. (Previously presented) The method of claim 30, wherein the recombinase-expressing cell is prepared by making an adenoviral E1-region protein-expressing cell additionally express a recombinase.

32. (Currently amended) The method of claim 23, wherein the recombinase-recognizing sequence in the ~~plasmid containing a~~ first therapeutic gene-expressing

plasmid ~~[[unit]]~~ is different from the recombinase-recognizing sequence in the proliferation-regulated plasmid.

33. (Previously presented) The method of claim 32, wherein the E1A region lacks a Rb protein-binding sequence.

34. (Currently amended) The method of claim 33, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region ~~and/or a 55KDa protein-coding region.~~

35. (Currently amended) The method of claim 23, wherein a drug tolerance gene in the proliferation-regulated plasmid and a drug tolerance gene in the first therapeutic gene-expressing ~~[[unit]]~~ plasmid are different from each other, and Ori in the first therapeutic gene-expressing ~~[[unit]]~~ plasmid can duplicate pir genes such as R6K γ only in competent cell.

36. (Previously presented) The method of claim 35, wherein the E1A region lacks a Rb protein-binding sequence.

37. (Currently amended) The method of claim 36, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region ~~and/or a 55KDa protein-coding region.~~

38-51. (Cancelled)